BIOBENEFICIATION OF BAUXITE ORE THROUGH BACTERIAL DESILICATION

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology

In

Biotechnology

By

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211BM2013

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2013

CERTIFICATE

This is to certify that the work in the thesis entitled " BIOBENEFICIATION OF BAUXITE ORE THROUGH BACTERIAL DESILICATION **"** submitted by Ms. Sahely Saha, in partial fulfillment of the requirements for the award of M. Tech (Biotechnology) at the National Institute of Technology Rourkela, is an authentic work performed by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any University/Institute for the award of any Degree or Diploma.

Prof. Krishna Pramanik Date:
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DECLARATION

The present study entitled "**Biobeneficiation Of Bauxite Ore through Bacterial Desilication"**is based on my original research work and no part of the thesis has so far been submitted for the award of degree in Master of Technology in Biotechnologyor any other degree or diplomato the **NIT Rourkela,** Orissa, India or elsewhere.

Place: Rourkela, Odisha

Date: **(Sahely Saha)**

ACKNOWLEDGEMENTS

This work was carried out at the Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Orissa, under the supervision of Prof. (Mrs.) Krishna Pramanik.

I would like to express my sincere gratitude to **Prof. (Mrs.) Krishna Pramanik** for her guidance and support throughout my work. Without her guidance, I would never have been able to complete my work with this ease. She was very patient towards the problems that I faced during the project work and helped me find the solutions. I am very much thankful to her for her valuable time.

I am very thankful to **Prof. Indranil Banerjee** for his valuable suggestions, advice and critical comments on my research work that helped me immensely in orchestrating different approaches to experimentation and in the assessment and interpretation of results.

I would like to express my sincere thanks to **Dr.Jayanta Kumar Patra**, Research Associate, **Mr. Devdutta Das**, **Ms. Rajdeep Kaur** and **Mr. Rama Raju Kuram** for their precious suggestions and encouragement to perform my project work. I thank God for giving me the chance to pursue my academic goals and a wonderful, complete life full of amazing people.

Finally, hearty thanks to my family member for their sacrifice that encouraged me to proceed with the work.

(Sahely Saha)

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ABBREVIATIONS

cm	-	Centimeter
ºC	-	Degree centigrade
et al.	-	And others
gm	-	Gram
hrs.	-	Hours
HCl	-	Hydrochloric acid
H_2O_2	-	Hydrogen peroxide
m	-	Meter
µg	-	Microgram
µl	-	Microlitre
mg	-	Milligram
ml	-	Millilitre
mm	-	Millimeter
mM	-	Millimolar
M	-	Molar
NA	-	Nutrient agar
H_2SO_4	-	Sulphuric acid
Wt.	-	Weight
w/w	-	Weight by weight
temp	-	Temperature
aeration	-	Initial aeration time
I size	-	Size of Inoculum
I age	-	Age of Inoculum
B conc	-	Percentage of bauxite

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ABSTRACT

Bauxite is an important mineral ore that is widely used in aluminum industry for metallurgical and refractory purposes. However bauxite contains silica as an impurity which degrades its quality. Silica forms complex with the caustic used during the processing of ore thereby forming precipitates. This leads to unnecessary wastage of caustic that contributes to the higher processing costs. Moreover, the use of excess caustic to neutralize the reactive silica during the process increases the alkalinity of the waste product so called red mud which imposes severe disposal problem. Therefore, the removal of silica from bauxite ore by a feasible and environmental friendly method is of paramount importance. The current study focuses on the beneficiation of low quality bauxite ores, through the process of bioleaching of silica. Bacterial desilication was carried out using indigenous bacterial cultures isolated from the ore. Bacterial colonies were successfully isolated and potential silica leaching strains was screened. Various process parameters such as pH, temperature, aeration time, inoculum size, age of the inoculum and bauxite percentage were studied through Taguchi method for process optimization. Optimum conditions for bioleaching of silica were obtained as pH 7.5, temperature 25ºC, initial aeration time of 30 minutes, bauxite percentage of 5% using 48 hours old 5% inoculums were established by Taguchi method,. Furthermore, optimum process conditions were used for bioleaching of silica under for different lengths of time i.e. 5, 15, 20, 25 days. Biological leaching results showed a maximum of 41% silica was recovered at the end of 25 days. Further, biochemical characterization of the potential bacterial culture proved it to be of *Bacillus* sp.

Keywords: Bauxite, silica, bioleaching, taguchi, bacillus sp.

CHAPTER 1

INTRODUCTION

CHAPTER 1

1. INTRODUCTION

Bauxite is the only ore of aluminum and hydrates of aluminum form the major part of its composition. According to the Mineral Commodity Summaries 2012, India occupies sixth position, in terms of World Bauxite Reserves. However silica is a major impurity present in bauxite ore that degrades its quality. Additionally, it forms complex with the caustic used during the processing of ore thereby forming precipitates. This leads to unnecessary wastage of caustic and undue shoot up of processing costs. Moreover, the use of caustic for neutralization of reactive silica during process increases the alkalinity of the waste sludge which causes severe disposal problem. Therefore, the removal of silica from bauxite ore is of paramount importance which can be beneficial to the aluminum industry to overcome the mentioned problems. Other established techniques, such as chemical methods, hydrometallurgy and pyrometallurgy, are non economic and release toxic byproducts. Therefore there has been an ever increasing demand for alternative methods to remove silica from bauxite.

The utilization of microbial consortia as well as its processes, to recover minerals from low grade ores has given a whole new dimension to the application of biotechnology. Metal extraction using micro-organisms is better known as bioleaching (Brierly,2008).With the incessant depletion of the high grade ores, there is a pressing need to unearth an economically viable method that enables us to exploit the low quality ores such as industrial wastes and mine effluents. Moreover, most of the products of bioleaching processes are in the form of aqueous

solutions which are much easier to treat and handle unlike the gaseous wastes. Therefore, the use of microbes for this purpose can address and resolve these technical barriers to a high degree.

The current study focuses on the beneficiation of low quality bauxite ores, through bacterial desilication . The objective is to reduce the amount of reactive silica with the help of microbial consortia. Apart from bioleaching, there are established chemical methods for processing bauxite ore such as screening and washing, gravity separation method, floatation method etc. each method has its own limitation such as low density difference between alumina and silica ; insufficient recovery of alumina and silica ,among others(Ehrlich et. al,2006). . Moreover, it is not advisable to use such capital consuming methods for the low quality ores with negligible recovery .

The rationale behind the undertaking of this project is to address a two- tier set of goals. Primarily, it focuses on the improvement of quality of bauxite ores. It makes the recovery of alumina easier and cost effective by significantly diminishing the usage of caustic soda used for the elimination of silicate impurities. On the other level, the eliminated silica also adds up value to the product chain because it finds application in a wide range of fields such as manufacture of ceramics and glass, for strengthening iron and steel, preparation of silica gel, also used as a moisture absorbent.

Objectives

The successful materialization of the concept of microbial desilication demands the isolation of novel strains of microorganisms, capable of desilication and the improvement of their activities either through process optimization or through introduction of modifications at the molecular

level. In tandem with the aforesaid objectives, the present piece of work was designed to study the following perspectives:

1. Isolation of indigenous bacteria with potential to leach out silica from the bauxite ore.
2. To evaluate the performance of isolated microbes in terms of silica removal from the ore.
3. Optimization of process parameters for silica removal, achieved by the selected bacterial strain.
4. Characterization of the isolated bacterial strain for its identification.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

2.REVIEW OF LITERATURE

In the recent age, there has been a perennial demand for biomining techniques globally, owing to various factors such as an upshot in the demand for metal commodities as a result of urbanization, shortage of key construction metals, scarcity of skilled labors considering the risks involved in the mining process and processing of polymeric minerals which is very cumbersome with the established methods, including smelting (Brierley ,2008).Therefore, novel technologies, for leaching has come into play.

The most commonly practiced methods of biomining include following techniques – whole ore leaching and concentrate leaching process. Bioleaching also deals with water purification via cyanide destruction process, heavy metal removal , nitrate removal, hydrocarbon removal. This chapter focuses on the advantages and shortcomings of all the existing, bioleaching techniques and also discusses the variations in consortium development according to the type of the reactors in use. It will also throw light on the existing lacunae at the industrial level that is hindering the successful commercialization of this technique. Moreover, the chapter focuses on the techniques of economic and environmentally amicable methods of silica removal from bauxite ore . The chapter also presents an overview of the hotspots of bauxite mining in India, importance of desilication of bauxite ore along with the future prospects of biomining.

2.1. Benefits of bioleaching

- Rich ores are on the verge of depletion, therefore it is essential to use and recover useful products from lower grade ores.

- Biomining demands lower capital investment whereas in conventional mining the operational costs are inversely proportional to the metal content of the ore.
- This process is suitable even for lower grade or complex ores.
- Bioleaching also allows the selective solublization of a particular mineral without effecting the others.
- Most of the byproducts and wastes are in liquid state which is much easier to manage than gaseous wastes. Moreover, there is scope for recycling of effluents.
- Can be used for leaching minerals present much beneath the earth's surface and is safer than conventional mining techniques.
- Use of indigenous microorganisms can cut the cost of buying bacterial, fungal strains.
- The process can be conducted in situ without the requirement of sophisticated technical support.
- The process environment friendly and save the company's expensive sulfur limits for emission.
- Reduced process control is required.
- Bioleaching is no more used just for the treatment of low grade ores, owing to its low economic requirements it has also been adopted in large scale processing of copper as well as pretreatment of refractory gold.

2.2.Established methods of mineral biomining and the technical challenges

The existing techniques which garner the major fraction of biomining industry include two methods- agitation and percolation . Both of these methods are used for whole ore processing as well as concentrate processing. Agitation is the method of using finer particles in a lixiviant. On

the other hand, percolation is considered more suitable for large scale operations. This procedure involves the flow of a leaching solution through a static bed, wherein the metal gets solubilized in the solution and seeps into the base due to gravity. On the commercial level two methods of percolation are in use.

2.2.1. Percolation methods

2.2.1.a.Dump bioleaching

This method is generally applied for the treatment of very low grade ores for which floatation and smelting are not cost effective. The ore is fractured by blasting and transferred from the pit to the dump. Acidified water is applied in the dump using drippers and sprinklers. When the solution percolates through the dump, ambient conditions for the growth of microorganisms is developed . These consortia catalyze the oxidation of minerals. Finally the mineral gets dissolved in the leach solution and is directed to the solvent extraction process from the base. The raffinate is recycled to the top of the dump(Rawlings, 1997).

It is considered to be a very successful method in terms of economic recovery of minerals from low grade ores such as that of copper. However,it is a time consuming method and leaching of copper is measured in decades because of the large particle size of the ores and inefficient percolation of the solution through the ores.With technical improvisation such as pre-conditioning of the ore with acidic ferric solutions, proper aeration, reducing the particle size by crushing this method is expected to give better results. Currently, Escondida mines in Chile are the largest dump biomining operation in the world .

2.2.1.b. Heap bioleaching

This technique is majorly used on a commercial scale for leaching of copper from secondary ores such as chalcocite (Cu2S) and covelite (CuS) ores and pretreatment of gold ores . Ore is crushed to 19mm or less and collected in rotating drums containing acidified water. The purpose of this step is to prepare the ore for the growth of acidophilic microbes and let the fine particles adhere to the larger rock particles. The ore is then stacked to a depth of 6-10 m over engineered leaching pads lined with high density polythene and perforated channels for drainage. On top of the drainage line a rock layer containing plastic air lines is placed. A blower is used to force air through these lines into the heap for the growth of microorganisms. Like dump bioleaching , the leached minerals get collected in the leaching solution and are removed from the bottom of leaching pad.

In case of gold ores , the fine gold particles are attached to the sulfidic minerals such as pyrite and arsenopyrite. Therefore, before dissolving gold through cyanide treatment, the sulfide is first oxidized . The pretreatment involves crushing of the ore followed by inoculation with three types of microorganisms such as thermophilic, extremophilic and mesophilic bacteria. Inoculation is performed on conveyer belts and stacked over the pads. Initial temperature for oxidation in the heap is 60ºC. The bacteria perform in succession along with the increase in temperature. Once the sulfide minerals get exhausted the temperature of the heap cools down. (Siddiqui M.H and Kumar A, 2009)

2.2.2. Agitation method

2.2.2.a. Bioleaching in stirred tanks

This method involves higher investments in terms of capital and operation costs but it is usually used for the recovery of precious metals. It is carried out in a series of continuous stirred tank reactors(CSTR) . Agitation is provided via impellers and aeration is supplied by blowers present below the agitator impeller. The walls of the tank consist of coils meant for the circulation of cooling water.

2.2.3. Vat leaching

This method is a combination of whole heap and stirred tank leaching. Sulphide mineral is first immersed in solution for the major part of the treatment. On the plus side, this method provides better control over bio-oxidation environment but the rate of reaction is low.

2.3. Consortium development for various types of reactors

Depending on the type of technique being used the environment for the growth of leaching microorganisms differ a lot and maintaining optimum conditions is another challenge .However, irrespective of the technique a good number of microorganisms are aerobic , inorganic and need low pH environment. They are mostly autotrophs and derive energy from the oxidation of sulfur and ferrous form of iron or both. In most cases the pH of the medium varies from 1.5-2. Iron and sulfur oxidizing bacteria are broadly distributed in the natural environment but these conditions do not provide the necessary optimum environment in a CSTR. Therefore, the primary requirement is to select such microorganisms which are able to adapt to the bioreactor conditions easily. It is already an established fact that bacteria isolated from a CSTR after a period of

growth is more efficient than the bacteria that were initially inoculated (Rawlings ,2005) . However, the time required for such an adaptation is unpredictable and might take a very long period. One such example was cited by (Rawlings , 2005) wherein the total residence time in a series of CSTR dropped from 7 days to 3.5 days over a period of three years but it was later extended to 4 days to rule out the scope of cell wash out. It was also discussed in (Mikkelson, 2006) that due to homogenous conditions in all the tanks only few species tend to dominate the entire niche inside the tank. For example ,in a case of chalcopyrite leaching Archae bacteria dominated the microbial population inside the tank ,under thermophillic conditions.

In case of heap reactors, ability of rapid cell division is not essential because chances of cell wash out are negligible. This can be attributed to the growth of microbial consortia over the mineral phase as a biofilm (Sand et al,1995 and Sand and Gehrke,2006) or dig into minerals (Rodriguez and Tributsch, 1988 and Edwards et. al, 2001)

2.4. **Alternative approaches for mineral bioleaching**

There are two strategies of developing microbial consortia for the dispensation of new minerals. The first approach involves the inoculation of a mixture of microbes and proceeds with the assumption that at least certain number of colonies will materialize as successful leaching agents. To assess the ability of these microorganisms , initially a lab scale leaching experiment is conducted in shaker incubator. After its accomplishment same work can be carried out in series of aerated tanks. This strategy is known as top down approach for mineral bioleaching However, for this approach it is necessary to have substantial microbial biodiversity in the initial inoculums so that the most efficient species can be selected. One additional advantage behind this rationale is that the large biodiversity of the inoculum makes it more vigorous as well as better adapted and robust to the operational changes.

The idea behind the alternative approach is to perform leaching experiment using 'logically designed' consortium in stirred tanks on the basis of operational parameters. Temperature and pH forms two of the major determinative factors. The microbial consortia should contain at least one iron oxidizer and one sulfur oxidizer. In most cases, *Leptospirillum ferriphilum* and *Acidothiobacillus caldus* is used for the purpose in the temperature range 35-45ºC. According to (Okibe et. al, 2003) heterotrophic acidophiles are also present in substantial number apart from the autotrophic iron/sulfur oxidizers which forms majority of the population. The role of these organisms is to metabolize organic materials which might otherwise inhibit some of the autotrophs (Johnson & Roberto 1997 and Okibe & Johnson 2004). Nonetheless, they also contribute in the mineral dissolution. The objective behind this concept is to find out the composition of the original micro flora and isolate various species of organisms present over the media. Possible combinations of different microorganisms are tested to find out the best combination for leaching, based on relative study of acid production , rate of mineral oxidation and leaching efficiency etc. (Johnson et. al, 2005).This method is useful when the number of microbial species in the stirred tank leachate is low. The method is advantageous because the consortium which does not play any role in mineral oxidation can be identified (Okibe et. al, 2003, Hugues et al, 2003, Gormely and Brannion, 1989).

2.5. Considerations while using 'top down' and 'bottom up' strategies

Two contrasting features have to be kept in mind while selecting the approach for experimentation i.e. adaptation for efficient growth v/s adaptation to a mineral The species to be used could either be "wild" type and therefore, be completely or partially adapted to the mineral or else could be taken from an already operational CSTR , therefore adapted for efficient

growth. It is extremely essential to strike a balance between this disparity depending upon the ability of the "wild" type to get adapted and the ease of the unadapted species to establish in the CSTR. Thus, it is advisable to use a growth efficient consortium, if available for similar kinds of mineral concentrates so that adaptation in the new mineral becomes easier and less time consuming. It is also necessary to consider and find out whether a single combination of micro-organisms is best suited for a particular bioleaching experiment or different combinations can replace each other as well.Once the most efficient combination is selected, the next task is to assess whether the efficacy would improve after selection in a CSTR or the species would be substituted by "wild" strains more adapted micro-organisms are less likely to be replaced. The "logical design " approach is particularly useful when the bioleaching conditions are beyond the conventional stirred tank operating conditions which in turn might be a limitation for the "wild" species. It has also been reported by that extreme acidophiles have much better tolerance and mineral dissolution capacity at extremely low pH than the already established consortium, being used on commercial level.

2.6. Bauxite ores

Bauxite is the only ore of aluminum and hydrates of aluminum form the major part of its composition. A large chunk of aluminum hydrate is present in the form of gibbsite, bohemite or diaspore. Most commonly the proportion of alumina in commercial bauxite varies from 40-60%, iron oxide in the form of Fe2O3 comprises the second large chunk of 7-30%,silica 1-15%, TiO2-3-4% along with other trace elements(Pradhan et.al ,1998). Several patents have been undertaken for the effective application of bauxite residue and its successful commercialization. Bauxite ore finds its maximum utility in metallurgy for the production of alumina. Other uses of the ore include abrasives, chemicals, refractories and cement industry. Chief sources of impurities

present in the ore are oxides of iron and silica which interferes with the recovery of alumina. The IS: 5953-1985(reaffirmed 2008), guidelines for metallurgical grade bauxite, state that the maximum permitted percentage of silica, is around 4% as mentioned in Table 1

TABLE1: Composition of bauxite ore

Constituent	**Grade I (%)**	**Grade II (%)**
Minimum Alumina	40	47
Available alumina(min)	36	43
Total Silica(max)	4	4
Fe2O3/TiO2(max)	30	30
Phosphorus pent oxide(max)	0.20	0.20
Vanadium pentoxide(max)	0.20	0.20

Data collected from Indian minerals year book 2011, 50th edition

2.6.1. Distribution of bauxite ore in India

According to the Mineral Commodity Summaries 2012, India occupies sixth position, in terms of World Bauxite Reserves Table 2.It accounts for around 6% of the global bauxite production. The highest number of bauxite resources (52%) are concentrated in Orissa, followed by Andhra Pradesh(18%), Gujarat (7%) , both Chhattisgarh and Maharashtra with 5% of the reserves, Madhya Pradesh and Jharkhand with 4% concentration. The commercialized ventures that supply bauxite as raw material to the aluminum industry have been listed out in Table 3.

TABLE 2. World bauxite reserves

Country	Reserves (In '000 tonnes)
Guinea	7400000
Australia	6200000
Brazil	3600000
Vietnam	2100000
Jamaica	2000000
India	900000
Guyana	850000
China	830000
Greece	600000
Suriname	580000
Venezuela	320000
Russia	200000
Kazakhstan	160000
USA	20000

Data collected from Mineral Commodity Summaries, 2012

TABLE 3: Aluminum industries and their sources of bauxite

Aluminum industry	**Source of bauxite supply**
NALCO	Mines at Koraput district in Orissa
BALCO	Kawardha dist. Mines in Chhatisgarh
HINDALCO (Renukoot) Belgaum	 Shahdol dist. In Madhya Pradesh Gumla and Lohardaga in Jharkhand Surguja dist. In Chhatisgarh Mines at Chandgarh and Kolhapur Dist.
Vedanta	GMDC Gujrat, Ashapura in Maharashtra

2.6.1. Types of bauxite ore

There are two major types of bauxite ores; lateritic and karst bauxite. Primarily, both are weathered products of aluminosilicate rocks. The former variety results from the leaching of silica from the aluminosilicate base resulting in deposition of valuable aluminous material on the top. On the basis of the age of deposit and weathering conditions , six different types of lateritic bauxite were categorized in (Bardossy and Aleva, 1990). The core silicate mineral is kaolinite which is generally associated with the iron mineral, goethite and gibbsite as the aluminous mineral. On the other hand, the latter is derived from rocks containing interbedded carbonate. Silicate minerals are in the form of kaolinite in this case as well, but are associated with aluminous minerals such as boehmite and diaspore. There are significant differences in the mineralogy of lateritic and karst bauxite deposits, which can be attributed to the different

oxidation and weathering conditions (Bardossy, 1982). Major concentration of karst and lateritic bauxite is located in Eastern Europe, Northern Asia and equatorial regions respectively.

2.6.2. PROCESSING OF BAUXITE ORE

The "processibility" of the bauxite ore largely depends upon the mineral composition . Lateritic bauxites are convenient to treat in comparison to karst bauxite. They demand less harsh temperature, caustic concentration and treatment time. The treatments of "high silica" versions of both the ores also vary accordingly (Peter Smith, 2009). Bayers process is the chief method for the processing of bauxite ore. Before, proceeding for the Bayers process, reactive silica is either removed or rendered inactive.

The key step for the production of Alumina involves treatment of the ore with caustic soda . This step is designed to separate out insoluble phases. Bauxite is first crushed and grinded which results in reduction of particle size and the solublization of kaolinite in the spent liquor. It is then transferred to the pressure digester via slurry storage. The insolubles are finally removed by "settling". The liquid is further filtered prior to precipitation. The caustic soda is recovered from the deposited insoluble mud and recycled back into the main process. Precipitation leads to the extraction of aluminum trihydroxide (hydrate). Hydrate crystals obtained, are passed through rotary calcinations kiln. It is during the calcination process that alumina is procured. The overall reactions have been demonstrated step wise in equations i, ii, and iii respectively.

Extraction

$$Al(OH)3 + Na^{+} + OH^{-} \rightarrow Al(OH)4^{-} + Na^{+} \quad \textbf{(i)}$$

Precipitation

$$Al(OH)4^- + Na^+ \rightarrow Al(OH)3 + Na^+ + OH^- \quad \textbf{(ii)}$$

Calcination

$$2Al(OH)3 \rightarrow Al2O3 + H2O \quad \textbf{(iii)}$$

After recovery of alumina bauxite is disposed in storage yards. The type of disposal depends upon various factors such as geographic conditions, environmental conditions, land availability, technology availability etc.

2.6.3. Types of bauxite disposal methods:

2.6.3.a. Dry Disposal

This method is adapted to reduce the land usage. It also prevents the seeping of leachate solution into ground water. It also reduces the rehabilitation costs significantly and the ore is in usable form. In this method the residue is washed and filtered using drum or press filters and a dry cake is produced. The dry cake is transported to the storage site without any further processing.

2.6.3.b. Mud stacking

This method is also known as slope deposition. It involves the thickening of the residue into a denser slurry containing around 48-55% solids followed by deposition and drying, before depositing successive layers. This method is beneficial in permitting the rain waters to run off and prevents leakage and provides structural stability to an extent. This method is an amalgamation of dry stacking and well drained depositon, that allows the recycling of reclaimed water and sodium salts.

2.6.3.c. Lagooning or ponding

The residue is stored in dams in the form of a dilute slurry. The presence of impervious layers at the bottom allows settling of solids with minimum leakage. The surface water is generally used for refining, after recycling.

2.6.3.d. Seawater Discharge

The method involved the disposal of bauxite residue into the sea followed by the caustic soda reduction treatments. However, this method is obsolete since 1970.

2.7. Bioleaching of bauxite ore

Silica is one of the major impurities present in bauxite ore that degrades its quality. Additionally, it forms complex with the caustic used during the treatment of ore thereby forming precipitates. This leads to unnecessary wastage of caustic and undue shoot up of treatment costs. The techniques of trimming the soda loss has majorly been classified under three broad categories. Firstly, through the pretreatment of the ore to diminish the amount of silica in the input stream. Secondly, through the alteration of the process which in turn would significantly reduce the production of soda residue or by recovering soda from the spent liquor. Various methods of reducing the concentration of reactive silica in the ore has been tabulated in Table 2.

TABLE4: Comparison of methods for reducing the concentration of reactive silica

Serial no.	Method	Description	Obstacle in commercial implementation
1	Screening & washing	"wet screens" or inclined screens are used to separate out small size pisolites or the fine silica rich layer adhering to larger pisolites	• Silica concentration on the outer surface of pisolite increases with the increase in surface area i.e with the decrease in size. Therefore size of pisolite is often a limiting factor. • Return of undersized particles to the minesite also poses a problem.
2	Gravity separation	Separation on the basis of density difference. Use of hydrocyclones to increase density difference.	• No commercial success so far. • Cannot be used when density difference is low. • Use of hydrocyclones shoot up the cost • Silica and alumina are mixed intimately. Therefore difficult to separate.
3	Flotation	Suitable chemicals are used to either activate the valuable minerals to the froth phase and separated from the gangue containing silica or else depress and deposit them at the base(reverse flotation)	• Technology might fail in case of lateritic bauxites wherein aluminum and silica liberation rate is low • Organic input related to the chemicals used , is also a limitation
4	Roast leaching	Ore is initially heated to 550º C to dehydrate kaolinite. Further heating at 980 º C to produce amorphous silica along with an intermediate of silica and alumina are formed	• Energy and cost intensive • Desilicated bauxite contains transition form of alumina instead of hydroxides of aluminum
5	Magnetic separation	Used for the separation of iron impurities. Generally used in conjugation with froth flotation process. Separation is based on two product streams.	• Can be implemented for removal of silica if reactive silica is associated with the iron fraction
6	Mechano – chemical treatment of bauxite with lime	It involves grinding of the ore which provides necessary energy for the chemical activation of the ore , leading to separation of insolubles.	• On addition of lime, hydrogamets are formed from silica as well as quartz . • Grinding demands high operational costs.
7	Bioleaching	A culture of microorganisms is used to solubilise and remove certain minerals for upgrading the quality and purity of ore.	• Time consumption is high • Non specificity of mineral solublisation • Separation of bacteria from desilicated bauxite

2.7.1. Microbes used in bauxite desilication and bioleaching

In majority of the cases studied so far, the solublization of bauxite depends upon the presence of organic acids in the medium. These organic acids are the products of microbial metabolism which either assist in the leaching of alumina from bauxite or solublization of the impurities, thus render a low quality ore into a higher quality. *Penicillium simplicisimum* and *Aspergilus niger* are among the fungal species which has demonstrated a crucial role in bioleaching of aluminum from bauxite (Ghorbani et. al., 2009 and Ambreen and Bhatti ,2002). The bacterial strain *Bacillus polymyxa* has been reported to be significant in calcium and iron removal from bauxite ore (Phalguni et. al , 1996). as described earlier bacterial strains *B. mucilaginosus* and *B. circulans* have demonstrated desilicating properties from bauxite ore (Wuxing Liu et. al,2006; Zhouhong-bo, 2006; Ehrlich et. al.,2009). The mechanism of microbial interaction with the silicates has been broadly summarized in (Ehrlich et. al.,2009; Friedrich et. al., 1991).

The three type of proposed mechanisms are

1. Annihilation of the crystal lattice of silicates
2. Bacterial uptake of silicon, for their metabolism- energy dependent uptake of silicon is evident in *Proteus mirabilis* in the presence of quartz(Heinen, 1965)
3. Release of silicon due to acid action

   It is already established that metabolic products such as acids (2- ketogluconic acid), ammonia, capsular slime of bacteria play a key role in dissolution of silicates (Webley et. al., 1960 and Duff et. al.,1963) . These metabolites either furnish protons that can destruct the Si-O bonds or can act as ligands capable of chelating cations from the crystalline structure, thus leading to its collapse.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER3

3.MATERIALS AND METHODS

3.1. Collection of sample and processing

Bauxite ore sample was collected from the storage yards of the aluminum industry, Hindalco Industries Limited, Muri works. The bauxite ore was dried at 100^0C for 24hours in hot air oven. Then it was powdered by using a mortar pestle.

3.2. Estimation of silica content in bauxite ore

Chemical analysis for estimating initial silica concentration in the bauxite was done. The bauxite ore was acid digested using sulfuric acid (Hurney, 1973). Then the solution was tested for the concentration of silica using ammonium molybdate method.

3.2.1Reagents

- Ammonium Molybdate solution – 5 gm ammonium molybdate was dissolved in distilled water and volume was made up to 50 ml. pH was adjusted between 7-8 using ammonium hydroxide.
- Oxalic acid- 5 gms oxalic acid dissolved in distilled water and volume was made up to 50 ml
- Hydrochloric acid 50%
- Merck standard silicon solution (1000 mg/L)

3.2.2 Standard estimation curve

- Various aliquots (10, 20, 30, 40, 50 mg/L) were prepared, using 100mg/L of standard silicon solution, for the calibration of standard curve.
- 25 ml of sample was taken in a flask and 0.5 ml HCl (50%) along with 1 ml ammonium molybdate solution was added to it.
- Color was allowed to develop by incubating at room temperature for 10 mins.
- Absorbance was taken at 410nm, using Systronics Double Beam Spectrophotometer 2203 with distilled water as blank.

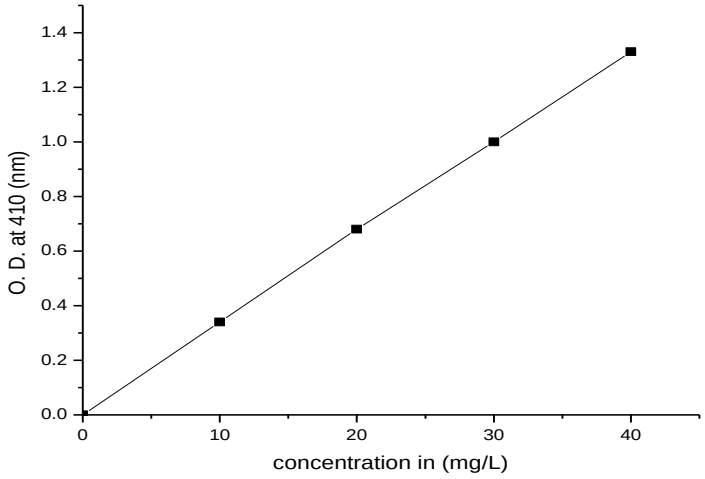

Fig 1.Standard curve for silica estimation

Calculations

% silica removal = (A/B)* 100

A= conc. of silica in 100 ml leachate solution after 7 days of treatment

B= conc. of silica in 100 ml culture containing 1 gm bauxite

3.3. Media preparation for isolation of microbial cultures

Suitable medium for the growth of silicate bacteria was prepared (Zhou Hong-bo et. al, 2006)

Media composition (g/L)

Growth medium-	
Sucrose	5
Na2HPO4	2
MgSO4.7H2O	0.5
FeCl3	0.005
CaCO3	0.1
Glass powder	1
pH	7.2

Bioleaching medium-	
Sucrose	5
Na2HPO4	2
(NH4)2SO4	1
MgSO4.7H2O	0.5
NaCl	0.1
CaCO3	1
Yeast extract	0.5
Bauxite ore	10
pH	7.2

3.4. Bacterial isolation and screening

Grounded ore powder was rinsed with distilled water. 1 gm of bauxite ore powder was dissolved in 9 ml distilled water and blended over magnetic stirrer followed by deposition for 20 mins.1ml suspension was taken and mixed with another 9ml sterilized water followed by serial dilution up to 10^{-6} times.10^{-4} to 10^{-6} dilutions were plated over solid growth medium and incubated for 48 hrs at 30ºC.The colonies were picked up from the solid medium plates and again sub cultured

over plates containing growth medium. Purification was repeated twice. Colony characteristics of the purified strains were observed.

3.5 Preliminary experiment for the selection of potential strain

Bioleaching experiment was performed with all of the isolated bacterial strains, to evaluate the desilication efficiency of each. The experiments were carried out in 250 ml plastic conical flasks, for 7days at 30 ºC in REMI orbital shaker, model no. 396 LAG, at 180 rpm. All experiments were conducted in triplicates.

3.6 Silica estimation using ammonium molybdate method

The leachate solution was filtered out using Whatman filter paper and silica concentration was estimated using ammonium molybdate method (Eaton, Celesceri and Greenberg,1999) .

3.7 To Study the growth kinetic of the bacteria

Growth kinetics of the bacteria was studied by measuring the OD, using Systronics Double Beam Spectrophotometer 2203. It was calibrated at 600 nm and growth media without inoculation was used as blank. 1 ml of bacterial suspension was centrifuged at 10000 rpm for 5 mins and the supernatant was decanted. Further, 1 ml distilled water was added to dissolve the pellet after washing it twice.Finally, volume was made up to 3ml and Optical density was read at 600 nm (at 0, 6 12, 18 , 24, 30, 36 hrs respectively).

3.8 To Study the effect of pH on bacterial growth

Growth Media was prepared as described in section 3.3 and autoclaved. Bacterial strain EBBO3 was inoculated in the medium and the culture was maintained at five different pH for 30ºC and 180 rpm. Finally, biomass was estimated by observing the absorbance using a spectrophotometer at 600nm after 48 hrs, using media as blank.

3.9 To Study the effect of different media on bacterial growth

Media optimization was carried out for the indigenous bacterial strain EBBO 3. Three different carbon sources such as sucrose, starch and dextrose were used for the purpose.50 ml growth media as described in section 3.3 was prepared in three separate conical flasks. 0.4 gm each of sucrose, starch and dextrose was used as carbon source. 2ml bacterial suspension was added to each of the three flasks and incubated for 48 hrs. To measure the absorbance, 1ml sample was collected from each and centrifuged at 10,000 rpm for 5mins. OD was measured at 600 nm using media as blank.

3.10 Optimization of process parameters influencing bioleaching of silica

Experiments for optimization of the removal of silica were carried out based on the following important parameters, using Taguchi method.

i) Temperature

ii) pH

iii) Aeration

iv) Bauxite concentration

v) Inoculum size

vi) Age of inoculums

3.11.a Design of Experiment using Taguchi Method

Taguchi method, or the Robust Design Method, introduced by Dr. Genechi Taguchi assists in improvising engineering productivity (Rao et. al., 2008). This method considers, the noise factors (environmental variation during the, manufacturing variation, product's usage and component deterioration) and assist the improvement of the primary functions of the process or th product, which in turn eases concurrent engineering and facilitating flexible designs. Indeed, it is the most powerful method available to reduce product cost, improve quality, and simultaneously reduce development interval.

Advantageous features-

A. Robust Design – a systematic, repeatable process.

B. Develop superior products at significantly lower cost in shorter time.

C. Quantify design reliability in dramatically less time and resources.

Taguchi's rule of three

Taguchi's rule comprises of three different stages-

1. Systems design
2. Parameter design
3. Tolerance design

Parameter design approach

Taguchi's approach provides the designer with a systematic and efficient approach for conducting experimentation to determine near optimum settings of design parameters for performance and cost. This method uses orthogonal arrays to aid the study of a large number of parameters with a small number of experiments. Orthogonal arrays reduces the number of experimental runs, hence saves time significantly (Ross P.J, 1988). The inferences obtained from small-scale experiments are applicable over the entire experimental surface covered by the control factors and their settings. Orthogonal arrays offer many benefit:

- The conclusion arrived from such experiments are valid over the entire experimental region spanned by the control factors and their settings.
- There is a large saving in the experimental effort.
- The data analysis is very easy.
- Easier to implement and can be applied if the number of control factors and their levels are known (Antony et. al, 1998)

This method can substantially cut the research and development costs through the simultaneous study of a large number of parameters. For the analysis of the results, this method utilizes a statistical measure of performance called signal-to- noise (S/N) ratio. Here, those factors which are not under operators control , are considered to be noise. The ultimate objective is to maximize the S/N ratio by eliminating noise. The S/N ratio considers both the mean and the variability.

The use of loss functions-For this purpose taguchi gave three situations along with three different equations.

Larger-the-better- It is used for the cases where the maximum occurence of a product characteristic is desired. Example- greater the rate of desilication, better is the quality of bauxite after treatment. Following S/N ratio is used.

η= -10 Log10 [mean of sum squares of reciprocal of measured data]

Smaller-the-better.-This situation is the reverse of the previous one. It is generally used to minimize the occurrence of defects.

The S/N ratio for such cases is-

η = -10 Log10 [mean of sum of squares of measured data]

Nominal-the-best

Here, a signal value (nominal value) is predetermined, and the variance around this value can be considered the result of noise factor. It is used when neither a larger nor a smaller value is desirable.

η=10 Log10(square of mean/ variance).

For the first set of experiments, a mixed level design with L18 array was analysed.SN ratio was calculated using larger the better approach.

η= -10 Log10 [mean of sum squares of reciprocal of amount of silica removed]

3.11.b Optimization of pH, temperature and aeration

Media was prepared using sucrose as Carbon source and separated out to maintain at various pH. Three levels of pH and two levels of aeration time were also varied in each experimental run. After 4 days of incubation the leachate solution was filtered out using 0.22 µm Whatman filter paper. Estimation of silica removed in the leachate solution was carried out using ammonium molybdate, method as mentioned in section 3.2 The aeration time was also co-related with the dissolved oxygen concentration of the media using Winkler's method. The SN ratios and the impact of each parameter on the overall response variable were analyzed and the optimal settings for pH ,temperature , aeration time were derived using Taguchi method. The three different parameters were analyzed using L18 orthogonal array with a mixed level design. All experiments were performed in triplicates and larger the better approach was adopted for SN ratio calculations.

Factors	**LEVEL1**	**LEVEL 2**	**LEVEL3**
Temperature (ºC)	**25**	**30**	**40**
pH	**6.5**	**7.5**	**8.5**
Aeration time(mins)	**0**	**30**	**-**

3.11.c Optimization of concentration of bauxite ore, size of inoculum, age of inoculum

Similarly, another set of experiments were performed, maintaining the optimal settings for pH, temperature and aeration, using a 2 level design with L8 array. All experiments were performed in triplicates. Parameters analysed in the second set has been tabulated.

Factors	LEVEL 1	LEVEL 2
Age of Inoculum(hrs)	24	48
Size of Inoculum(%)	5	10
Bauxite Concentration (%)	1	5

3.12 Optimization of treatment time

The desilication experiment was carried out maintaining optimal settings of all the parameters obtained and percentage of silica removed in the leachate solution was analyzed at intervals of 7days, 15 days, 20 days, 25 days. Finally, pH of the residue after the treatment was also recorded.

3.13 Analysis of the treated bauxite ore

Analysis of both the samples (i.e. before and after treatment) with the isolated bacteria, was performed using Scanning Electron Microscope. In both the cases the finely grounded samples were mounted over SEM stubs to view the surface morphology. Samples were coated with platinum , prior to analysis . This step was performed to aid the generation of secondary electrons, back scattered electrons, diffracted scattered electrons etc. from a non conducting surface. SEM (JEOL-JSM 6480 LV) at 15 kV voltage, at 5000X magnification was used for the above mentioned purpose.

3.14. Characterization and identification of the bacterial strain EBBO3

3.14. a SEM analysis of bacterial strain

A smear of bacterial sample was prepared over the stub and air dried. Smear was dried overnight after treating with 0.25% glutaraldehyde. Heat fixing was followed by washing with buffer. Varying concentrations of ethanol (30%, 50%,70%, 80%, 90%, 100%) was used for dehydration of the cells. After addition of 100% ethanol, the sample was incubated for 1 hour. Cells were coated with platinum and observed using SEM (JEOL-JSM 6480 LV) at 15 kV voltage,using magnification of 5000X and 10000 X .

3.14.b Biochemical characterization of the bacteria

Biochemical tests were carried out according to (Cappuccino and Sherman, 2002), Microbiology: A laboratory manual.

Gram staining

Smear was prepared by isolating bacterial strains from the petridish and mixed with a drop of distilled water, with a wire loop. Smear was heat fixed, once air dried. Smear was stained with crystal violet and washed with distilled water after 1min.Few drops of grams iodine was added and allowed to stand for 1min, followed by a wash with distilled water. Washing was repeated in absolute ethanol for about 15 seconds. Smear was again washed in distilled water and stained with safranin. Samples were viewed microscopically after washing and drying.

Catalase test

20ml nutrient agar was prepared and allowed to solidify on a petridish. A bacterial colony was picked using a wire loop and spread over the agar plate, in a circular manner. A drop of hydrogen peroxide was poured over the bacterial colony.

Urease test

Urea was added into urease broth, using a syringe filter. Bacterial culture was inoculated in the broth and incubated at 30 ± 1º C for 72 hrs. The test detects the presence of urease enzyme in the test micro organism. Urease enzyme splits urea into ammonia and carbon dioxide, which is indicated by adding a few drops of Phenol red indicator.

Composition	**grams/litre**
Peptone	1
NaCl	5
K_2HPO_4	2
Glucose	1
Urea	20
pH	6.8
Phenol red	6 ml

Oxidase test

The test detects the ability of bacteria to produce enzyme oxidase**.** Few drops of methylene blue was added to the 72 hr old bacterial culture maintained at 37 º C. The change in color of the dye from dark blue to colorless, indicates the bacteria produces oxidase enzyme.

Citrate utilization test

The test determines the ability of the micro organism to utilize citrate present in the medium, as their sole carbon source. In case of citrate utilization the pH of the medium rises from 6.8 and the change in pH is accompanied by the change in color of bromothymol blue indicator. The bacteria was inoculated in Simons citrate medium and incubated for 48 hrs. the deep blue color development indicates a positive test. On the other hand, persistence of yellow color shows a negative test.

Methyl red test

The test organism was inoculated into 5ml of MR broth and incubated at 37 º C for 72 hrs. Few drops of methyl red was added to 1ml of the broth. A development of red color signified a positive MR test and on the other hand a yellow color suggested a negative test.

Indole test

1% tryptophan broth was inoculated with a bacterial colony and incubated for 37 º C . After 48 hrs of incubation , approximately half the total volume of Salkowsky's reagent was added and shaken gently.

Salkowsky's Reagent-1ml 0.5M FeCl3 added to 50 ml of 35% perchloric acid. The tube was shaken vigorously after adding 1 ml chloroform to the above solution

Carbohydrate acid gas test

Two 100ml nutrient broth cultures were prepared separately and 1% dextrose and sucrose were added to first and second culture respectively, after autoclaving.6ml bromothymol blue indicator was added to both the cultures.8 ml of broth from each culture was inoculated. Durham tube was

completely filled with media to remove any air bubble present. It was then inverted into the test tube till it immersed completely.

Nitrate reductase test

Nutrient broth was prepared along with 0.2% of potassium nitrate and autoclaved. Bacterial was inoculated and incubated at 30 ºC for 96 hours. A few drops of 1:1 mixture of sulfamilic acid and α naphthol was added to the incubated culture.

Reagents -Sulfamilic acid (0.2 gm in 25 ml 5N acetic acid)

α naphthol(0.25 gms in 50 ml 5N acetic acid)

Voges Proskauer test

The test is also known as acetoin test as it detects the potential of bacteria to ferment carbohydrates such as glucose and produce end product acetylmethylcarbinol (acetoin). For the purpose, bacterial culture was inoculated in nutrient broth and incubated at 30 ±1 ºC for 72 hours.A mixture of 3ml α naphthol was mixed with 1ml of 40% KOH.

Dihydrogen sulfide gas production test

1% Ferric chloride was added to nutrient broth and autoclaved.Filter paper was saturated with lead acetate solution and introduced inside the test tube containing culture.The culture was maintained at 30 ºC, undisturbed for 7 days.

Starch hydrolysis test

The bacteria was inoculated on NA plate added with 1% starch solution and incubated at 30°C for 24 hrs. the plates were flooded with iodine solution for five minutes and excess solution was decanted and starch hydrolysis was noted from a clear zone formed around the colonies. Reddish brown area around colonies indicated partial hydrolysis of starch. The diameter of the clear zone was measured using antibiotic inhibition zone scale and the ratio calculated from diameter from the clear zone and diameter of the bacterial growth gave the activity level.

Antibiotic assay

Response of the organism to different antibiotics was tested on NA medium. NA plates were surface seeded with concentrated bacterial suspension. Different antibiotic discs with effective concentrations were place over the plates. Inhibition of growth depicted by a clear zone formation around the discs indicated sensitive reaction otherwise the organism was resistant to the antibiotic. Diameter of the inhibition zone was measured with an antibiotic zone scale. Ratio of the inhibition zone and disc area produced the activity level of the antibiotic.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

4. RESULT AND DISCUSSION

4.1. Estimation of silica in the bauxite ore before treatment

It was estimated that, roughly 4% silica was present in bauxite ore before treatment.

4.2. Bacterial isolation and screening

Five different colonies were selected and pure cultures were obtained after two subcultures.

Colony characteristics have been tabulated in TABLE 5

TABLE 5: Colony characteristics of the isolated bacteria

Colony characteristics	**EBBO1**	**EBBO2**	**EBBO3**	**EBBO4**	**EBBO5**
Color	Translucent	Yellowish-Off white	Off white	Milky white	Off white
Shape	Circular	Circular	Oval-circular	Circular	Circular
Texture	Very sticky (gummy)	Less sticky and less viscous	Less viscous	Non gummy	Less viscous
Elevation	Convex	Flat	Less convex	Less convex	Convex
Population	Dense	Dense	Dense	Isolated colonies	Isolated colonies

4.3. Selection of potential silica removing strain

The percentage of silica leached by each bacterial strain has been tabulated in TABLE 6. It is evident that maximum percentage of silica was removed by the bacterial strain EBBO3. The treatment with strain EBBO3 was found to leach silica 20 times more than the control. Hence, the strain EBBO3 was found to be the most efficient strain achieving maximum amount of silica removal and thus was selected for further bioleaching study.

TABLE 6: Table showing percentage of silica leached by each bacterial strain

Sr no.	Treatment	Concentration of silica removed (mg/L)	Percentage of silica removed
1	EBBO 1	8	2
2	EBBO 2	16	4
3	EBBO 3	70	17.5
4	EBBO 4	5	1.25
5	EBBO 5	4	1
6	Control	3.5	0.08

4.4. Study of growth kinetics of the bacterial strain EBBO3

It is evident from the graph that the growth curve of bacteria attained its log phase nearly after 12 hours of incubation and the growth was steady after a period of nearly 30 hrs of incubation.

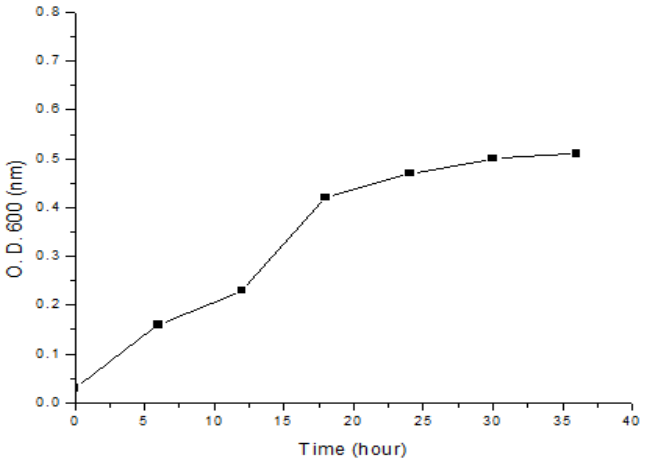

Fig 2. Growth curve of bacteria EBBO3

4.5. Effect of different pH on the growth of bacterial strain EBBO3

It is indicated from the bar graph that maximum biomass was obtained at pH 7.5 followed by pH 7. And minimum biomass pH 6 biomass was observed at 600nm.

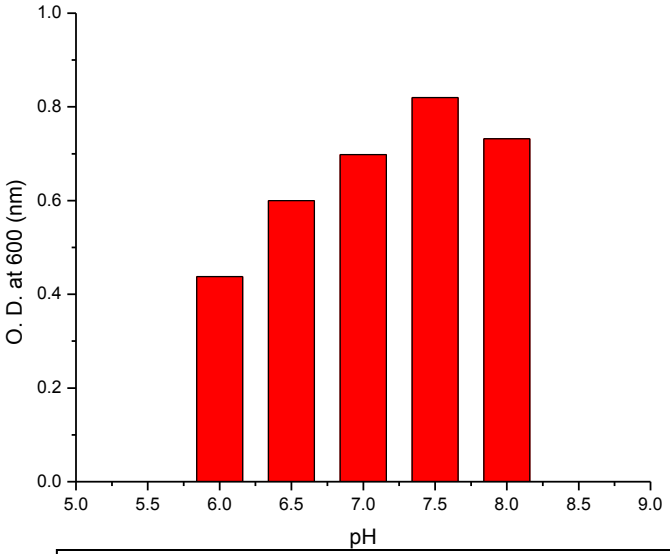

Fig 3. Effect of different pH on the growth of bacterial strain EBBO3

4.6. Effect of various Media on bacterial growth.

The graph suggests that maximum growth of isolated bacteria EBBO3 was observed when sucrose was used as carbon source. It was followed by starch and dextrose.

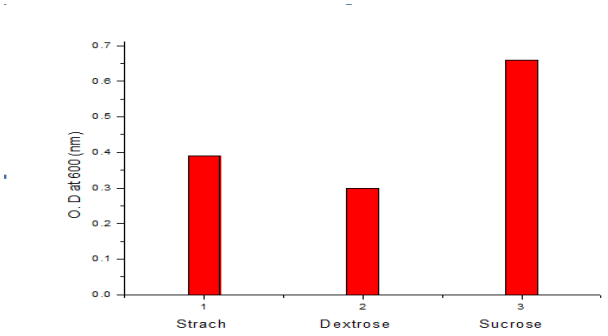

Fig 4. Media optimization

4.7.a Optimization of pH, Temperature and aeration time

The first set of bioleaching experiments were conducted using initial pH of the medium, temperature and aeration time as factors influencing desilication. Both pH and temperature are highly essential for the growth of the desilicating bacteria. Moreover, pH plays a crucial role in metal solublization. Additionally, ample oxygen supply is also necessary for the activity of bacteria (Bosecker K, 1997). Therefore, the initial dissolved oxygen concentration in the autoclaved media, aerated for 0 mins and 30 mins, were estimated through Winkler's method(REF) and found out to be 2 mg/L and 8mg/L respectively.

Desilication experiment was followed by the estimation of silica removed through ammonium molybdate method. The experiments were performed in triplicates and observations have been tabulated in the TABLE 7.

TABLE 7: Effect of pH, temperature and aeration time on silica removal

Sr. no.	Temp(°C)	pH	Aeration(mins)	OD at 610 nm			Conc. of silica in solution(mg/L)		
1	25	6.5	30	1.25	1.34	1.14	31	40	30
2			0	0.7	0.7	0.58	21	21	17
3		7.5	30	3.07	1.36	1.00	93	42	30
4			0	1.31	1.34	0.9	32	40	28
5		8.5	30	1.01	1.11	1.00	30	30	30
6			0	1.00	0.86	0.85	30	25	31
7	30	6.5	30	1.26	0.82	1.67	40	25	52
8			0	0.11	0.10	0.43	3	3	12
9		7.5	30	3.07	2.75	2.35	93	85	74
10			0	0.59	0.52	3.07	17	15	93
11		8.5	30	0.86	0.44	0.49	26	14	15
12			0	1.00	1.36	1.66	30	42	51
13	40	6.5	30	0.31	0.11	0.34	9	2	10
14			0	3.07	0.42	1.97	98	12	62
15		7.5	30	2.2	2.6	1.45	67	80	44
16			0	0.5	1.35	0.5	15	40	12
17		8.5	30	0.5	0.93	0.11	13	27	2
18			0	0.4	0.4	0.5	13	13	15

Analysis of the responses through Taguchi design

The results obtained after optimization were analyzed using Taguchi DOE through MINITAB 16 software. The TABLE 8 shows the use of L18 orthogonal array for the aforesaid purpose along with the SN ratios.

TABLE 8: L 18 orthogonal array for optimization of pH, temperature and initial aeration time in relation to silica bioleaching.

↓	C1	C2	C3	C4	C5	C6	C7
	aeration	Temp	pH	D1	D2	D3	SNRA1
1	0	25	6.5	21	21	17	25.7428
2	0	25	7.5	32	40	28	30.1817
3	0	25	8.5	30	25	31	29.0289
4	0	30	6.5	3	3	12	11.1697
5	0	30	7.5	17	15	93	25.7293
6	0	30	8.5	30	42	51	31.6273
7	0	40	6.5	98	12	62	26.1328
8	0	40	7.5	15	12	40	23.9744
9	0	40	8.5	13	13	15	22.6550
10	30	25	6.5	31	40	30	30.3359
11	30	25	7.5	93	42	30	32.2339
12	30	25	8.5	30	30	30	29.5424
13	30	30	6.5	40	25	52	30.6301
14	30	30	7.5	93	85	74	38.3699
15	30	30	8.5	26	14	15	24.3471
16	30	40	6.5	9	2	10	10.4200
17	30	40	7.5	67	80	45	35.3618
18	30	40	8.5	12	27	2	10.6497

Based on the SN ratios a response table was generated. The TABLE 9 indicates the ranks and delta values for each factor. Here, the delta values signify the size of effect of each characteristic (i.e. the difference between the highest and lowest values for each response). It can be deduced from the table that the SN ratio, hence the response is effected by all the three factors in the order pH> temperature>aeration. It is also evident from the table that level1, level 2 and level2 of temperature, pH and aeration time respectively has the greatest impact on the response.

Table 9: Response table for pH, Temperature, Aeration

Level	Temp	pH	aeration
1	29.51	22.41	25.14
2	26.98	30.98	26.88
3	21.53	24.64	
Delta	7.98	8.57	1.74
Rank	2	1	3

MAIN EFFECT PLOTS

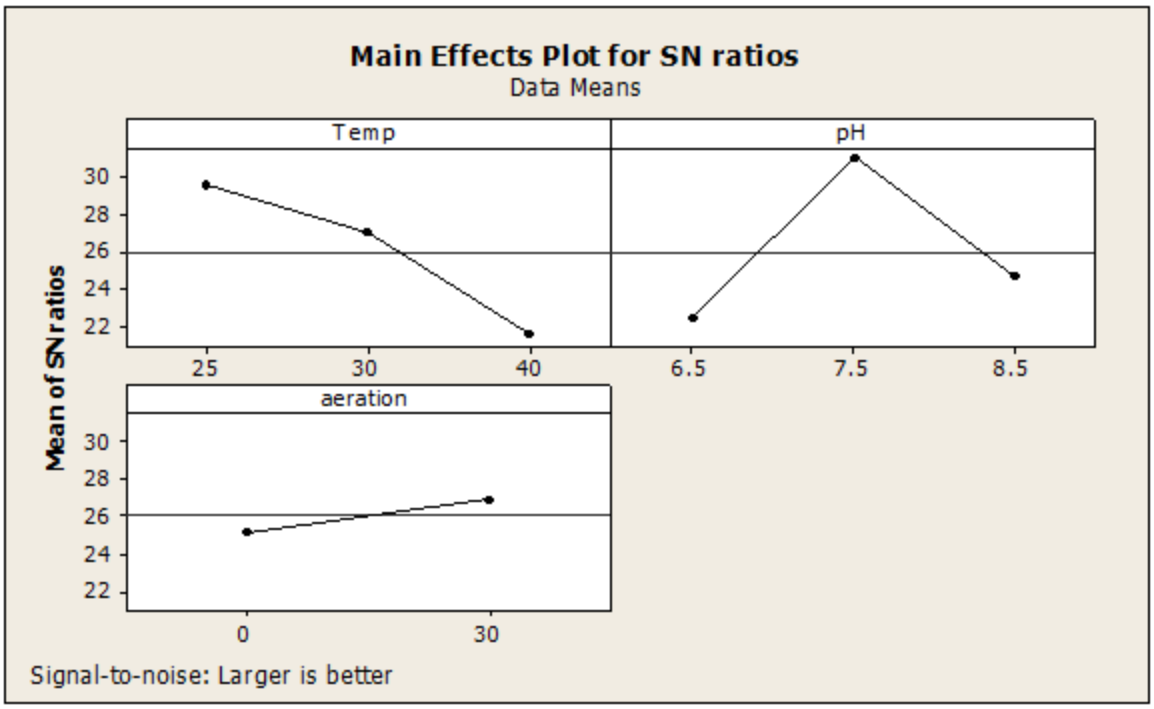

Fig. 5 Main effect plots for temperature, pH and aeration

The main effects plot signifies the impact of each factor on the response characteristic. The slope of the line joining the two levels, points out the presence or absence of main effect (i.e. every point present on the line depicts the effect of the specific factor level, on the response characteristic). Based on this plot, it can be suggested

- Maximum impact on the response variable was observed at a temperature of 25°C followed by 30°C which indicates the bacteria EBBO3 is mesophilic. A sharp drop in bioleaching potential of the bacteria was observed at temperatures nearing 40° C. These observations are in line with that of bacterial strain Lv1-2 (Zhou Hong-bo et. al, 2006).
- The desilication was high in the range of pH 7 to 8. The highest response was recorded at pH 7.5. However, the strength of response dropped near pH 8.5. Lowest response was observed at pH 6.5. The results obtained are similar to the bacterial strain Lv1-2. (Zhou Hong-bo et. al, 2006).
- The gentle change in response curve from 0 mins of initial aeration (2mg/L DO) to 30mins of initial aeration (8mg/L DO), depicts that the aeration had a mild impact on response. The result is justifiable, as the DO concentration could not be monitored and maintained throughout the incubation period, on a shaker incubator level. Moreover, the interaction with other factors are also responsible for the observed response. However, the parameter might be of vital importance for carrying the desilication experiment on the reactor level.

INTERACTION PLOTS

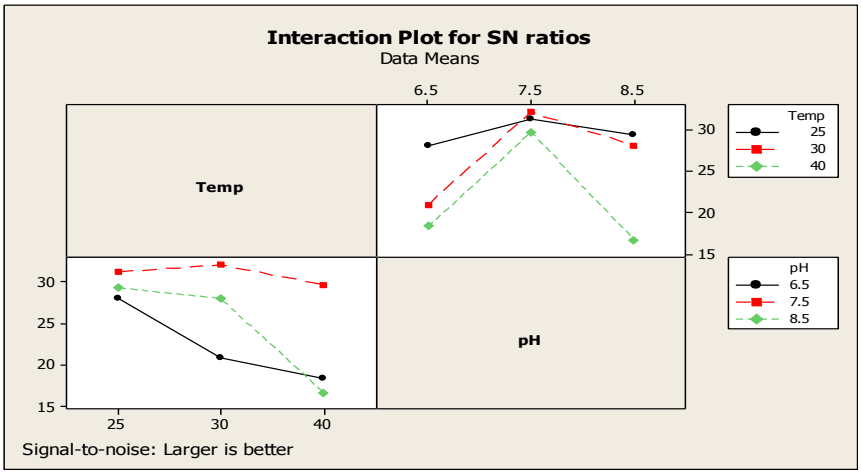

Fig 6. Interaction between temperature and pH

The interaction plot between temperature and pH suggests that both the variables are highly interacting. However, it is evident from the column1 graph that pH 7.5 has the highest influence on the response in the entire temperature range of 25°C to 40°C.

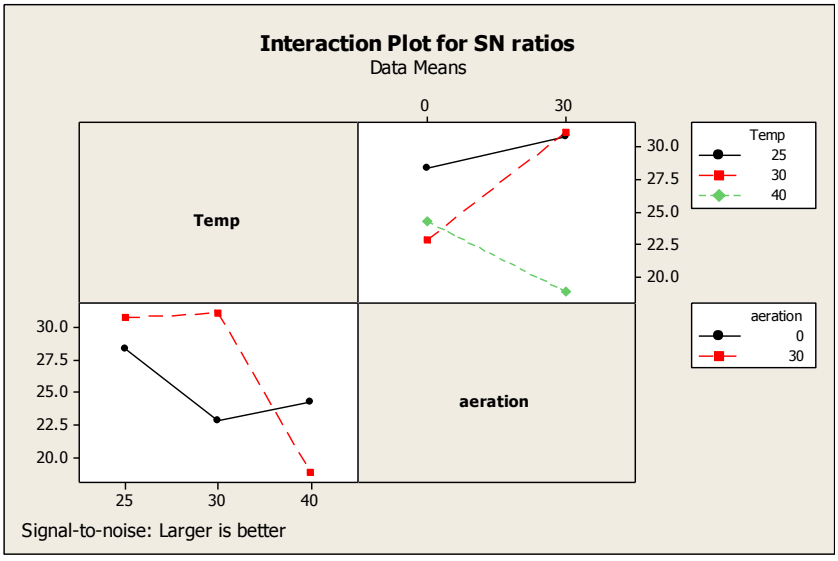

Fig 7 Interaction between Temperature and aeration

From the interaction between temperature and aeration time, it can be derived that desilication is highest at 25°C , both in presence or absence of aeration. RHS graph suggests, in the temperature range of 25°C- 30°C the SN ratio, hence the response is better with 30 mins aeration.

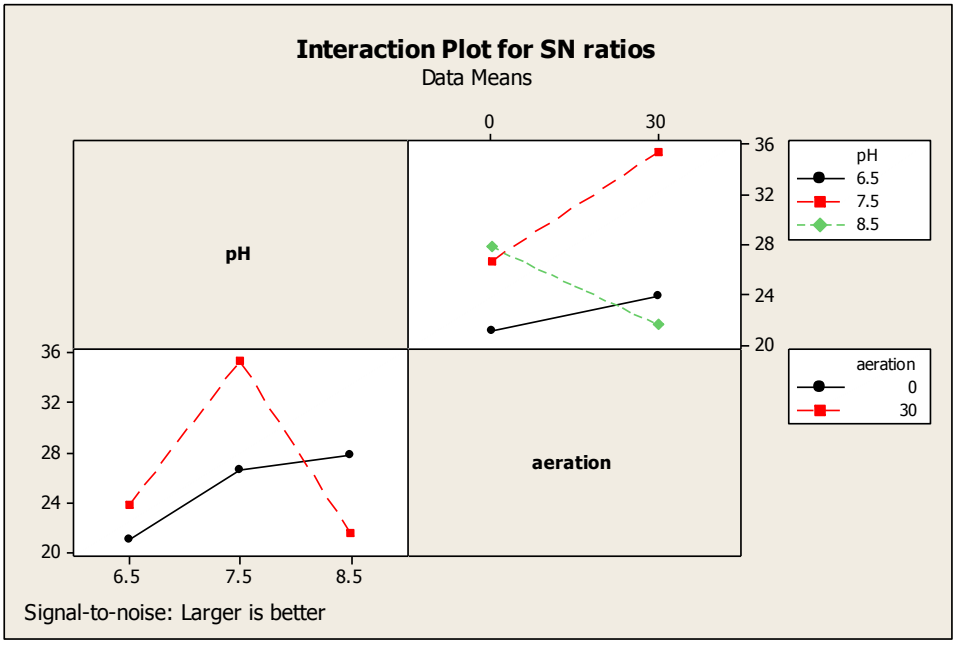

Fig 8. Interaction between pH and aeration

Both pH and aeration, hence Dissolved Oxygen are interacting factors. LHS graph depicts the response is lowest around pH 6.5, in case of aerated as well as non aerated media. On the other hand , the SN ratio hence Desilication response is on the higher side around pH 7.5, irrespective of aeration. LHS graph , indicates, the interaction between the two factors at pH 6.5 and 7.5 is meek. The aforesaid observation is supported by the almost parallel lines in the graph

NORMAL PROBABILITY PLOTS

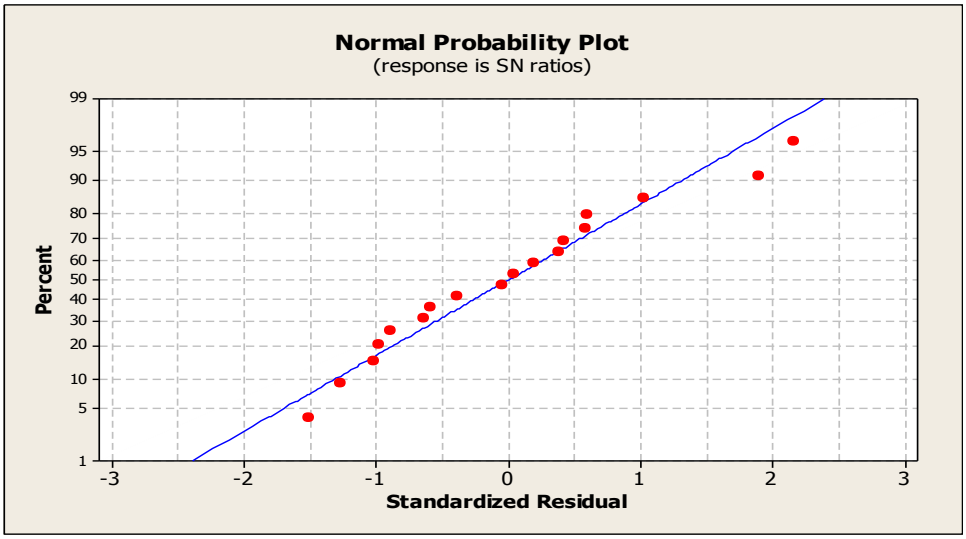

Fig 9. Normal probability plot for pH, temperature and aeration

Residuals depict the difference between observed and fitted response values whereas Fitted value is the predicted response value. It is calculated using regression equation.It is evident from all the normal probability plots that the response roughly follows the straight line. Hence, the residuals indicate normal distribution.

4.7.b Optimization of inoculum size, age of the inoculum and percentage of bauxite

From the analyzed data, optimum settings for pH, temperature and aeration were obtained. The next set of optimization studies for Inoculum size, Inoculum Age and bauxite concentration were conducted using pH-7.5, temperature 25°C and aeration for 30 mins.

TABLE 10. Response table for inoculum size, age of inoculum and bauxite percentage

Response Table for Signal to Noise Ratios
Larger is better

Level	I age	I size	B conc
1	23.46	25.81	24.66
2	26.31	23.96	25.11
Delta	2.86	1.85	0.46
Rank	1	2	3

It is evident from the rank and delta values that inoculum age is the factor with greatest effect on percentage of desilication, followed by Inoculum size and Bauxite concentration in descending order. It is also clear from the response table that level 2 of factors, inoculum size and bauxite concentration have a higher S/N ratio and indicates a greater impact. On the other hand, level 1 of inoculums size indicates a greater impact on the response variable.

MAIN EFFECT PLOTS

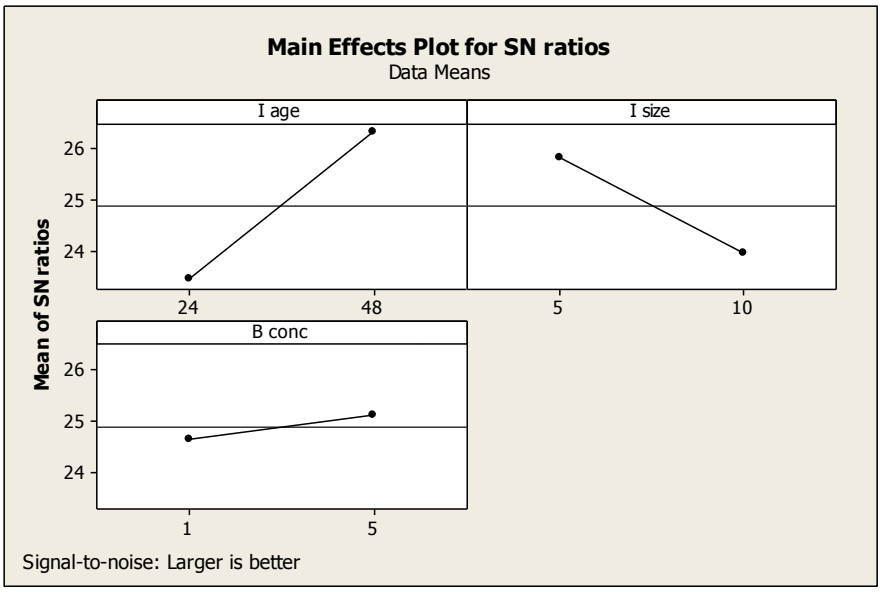

Fig.10. Main effect plots for inoculum age, inoculum size and bauxite

This plot indicates, Inoculum age has the highest main effect on the S/N ratio followed by Inoculum size. Bauxite concentration has very low main effect as the slope of the line is negligible.

- The 48 hr old culture exhibited a higher impact on the response. It can be supported with the observation in the growth curve, the bacterial strain EBBO3 completes its growth in more than 24 hours.

INTERACTION PLOTS

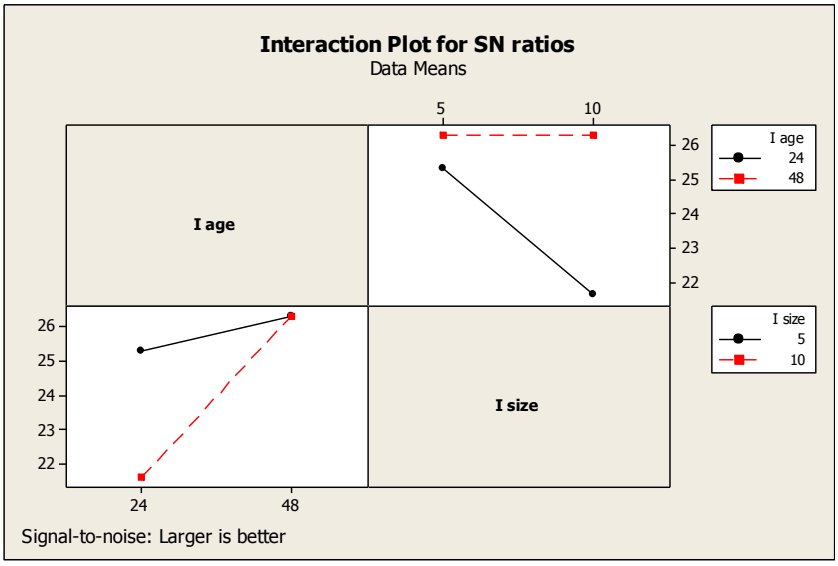

Fig 11. Interaction between inoculum age and inoculum size

In the graph, the interaction between factors Inoculum age and Inoculum size has been depicted. Column 1 indicates, both Inoculum sizes i.e. 5% and 10% gives better response with a 48 hr old culture. On the other hand, column 2 suggests, 24 hr old culture gives much greater SN ratio than at 5% Inoculum size, while the 48 hr old culture does not exhibit any difference at both 5% and 10% I size.

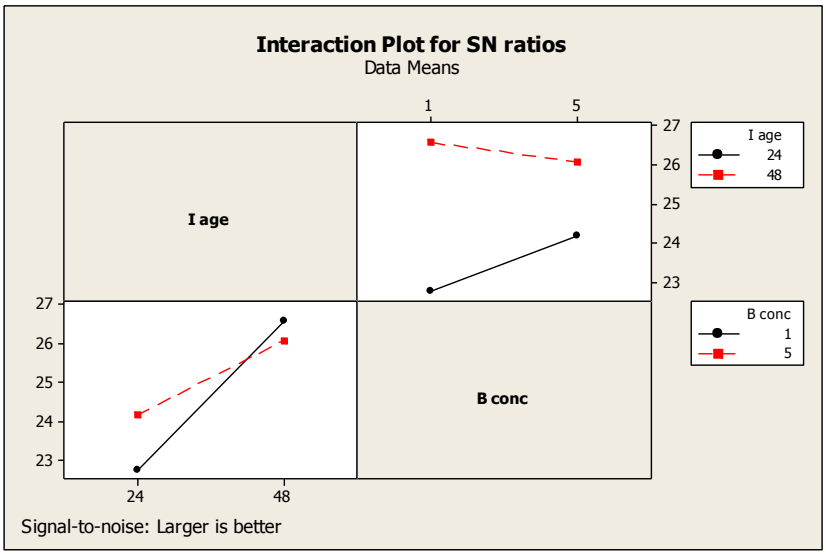

Fig 12 interaction between inoculum age and bauxite concentration

Column 1 represents that with 1% and 5% bauxite concentrations, the desilication percentage nearly constant when a 48 hr old culture is used.Column 2, indicates slightly higher desilication is obtained with 1% bauxite concentration, using a 48 hr old culture, while the 24 hr old culture depicts a slightly better response with 5% of initial bauxite concentration.

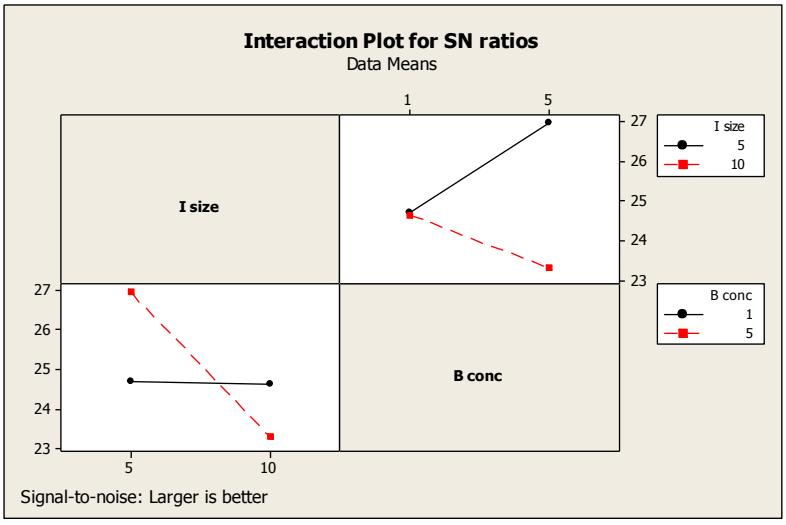

Fig 13. Interaction between inoculum size and bauxite concentration

Both the columns depict high interaction between Inoculum size and bauxite concentration. Column2, indicates response is high at 5% bauxite concentration, with 5%inoculum size. Similarly, column1 suggests a high response when bauxite percentage is 5% and inoculum size is also 5%.

NORMAL PROBABILITY PLOTS

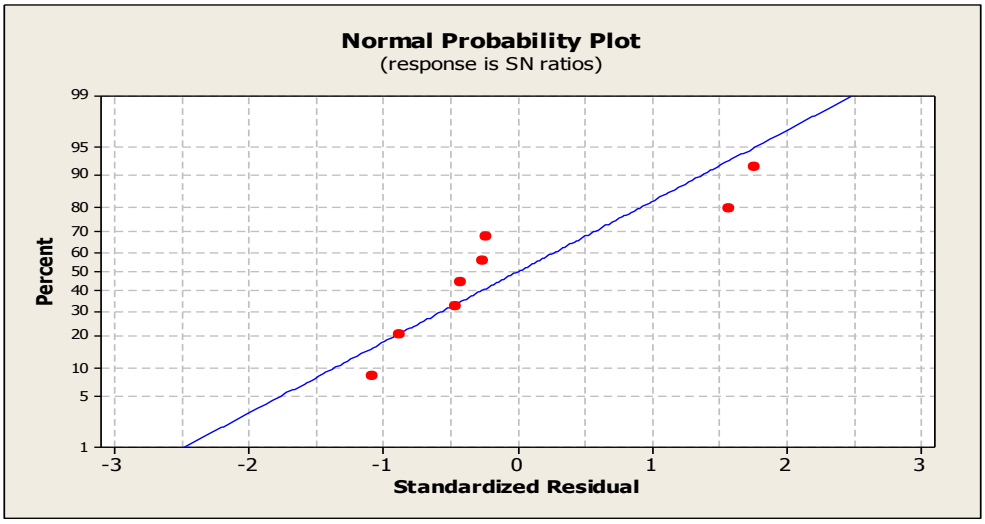

Fig 15 Normal probability plots for SN ratios for I age, I size and B conc. optimization

The normal probability plot indicates normal distribution with moderate fluctuations, in this case of balanced design of factors.

4.8 Optimization of treatment time

The desilication experiment was carried out for a period of 25 days. It was observed that percentage of desilication was 25%, 33%, 38% and 41% at 7 days, 15 days, 20 days and 25 days respectively. Thus, it is evident from the results that maximum desilication percentage was

obtained after 20 days which is almost stable by 25 days. After the treatment the change in pH was also recorded. pH varied from initial 7.5 to 6.2, 5.8, 5.6 and 5.5 after the 7^{th}, 15^{th}, 20^{th} and the 25^{th} day respectively.

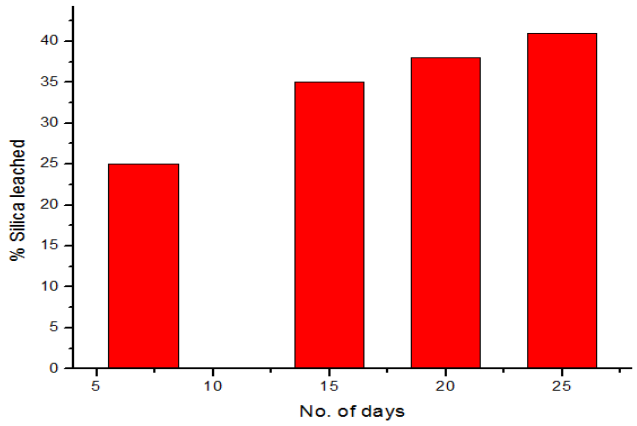

Fig 16. Optimization of treatment time

4.9. SEM analysis of the bauxite ore before and after the treatment with EBBO3

A significant reduction in the particle size of the ore, was observed, after the bacterial treatment for 7 days.

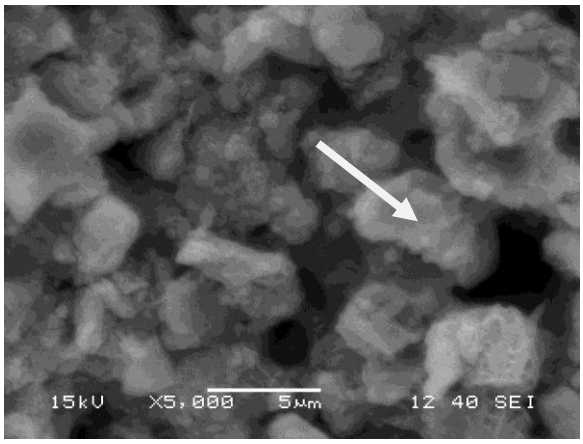

Fig 17a. Larger particle size before treatment

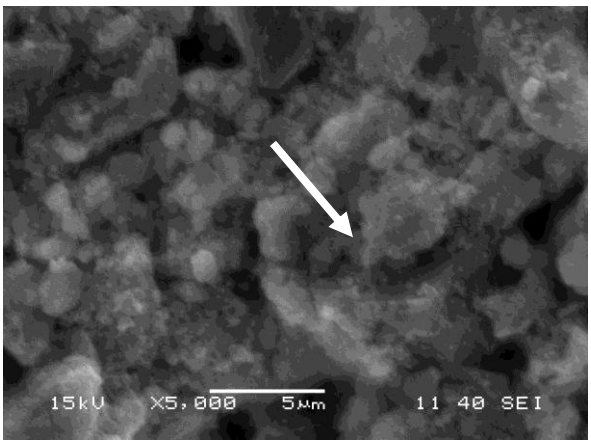

Fig.17 b. Smaller particle size after treatment

4.10. Characterization and identification of the isolated bacteria EBBO3

4.10.a SEM characterization of the bacteria

It is evident from the SEM images (Fig 20.A & B), the bacterial strain EBBO3 is rod shaped with a size 1.1-21µm X 912nm.

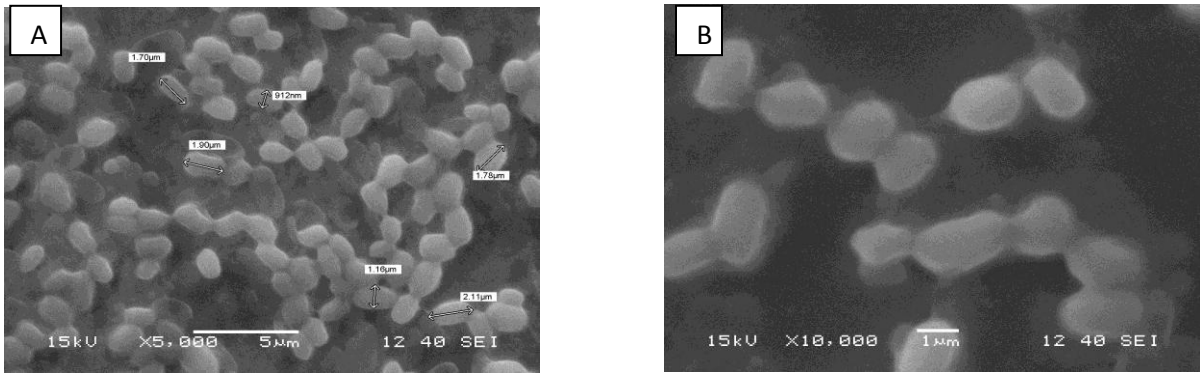

Fig 18 a&b. SEM observation of bacterial strain EBBO3

4.10.b Biochemical characterization of the bacteria

Gram staining

The microscopic view of the isolated bacterial strain depicts , EBBO3 is a large and uniform Gram positive bacilli.

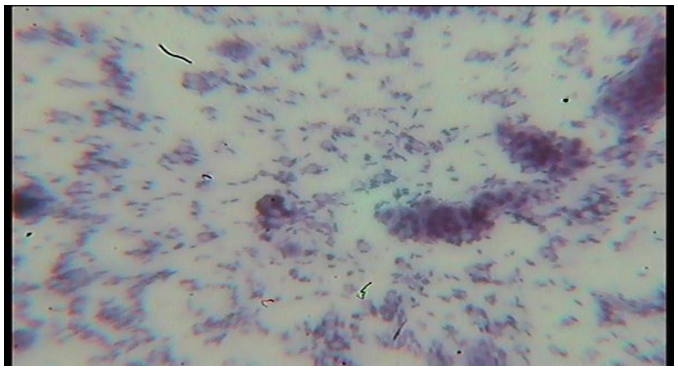

Fig 19 Gram staining shows bacteria EBBO3 s gram positive

Catalase test

In the presence of catalase producing microorganisms, hydrogen peroxide splits into water and oxygen, leading to the formation of bubbles. No bubble formation on addition of hydrogen peroxide suggests the bacterial strain EBBO3 is catalase negative.

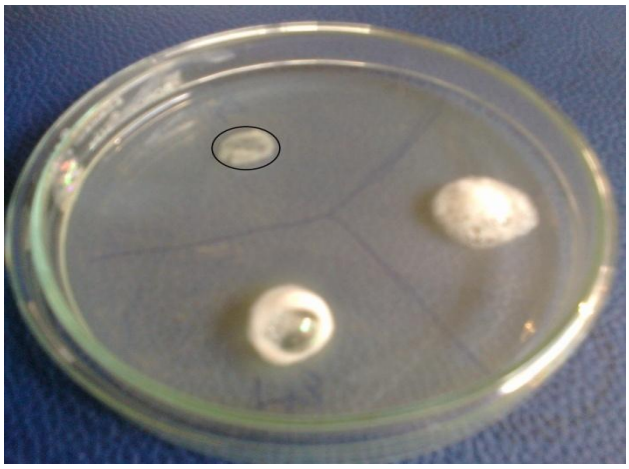

Fig 20. Bacterial strain EBBO is catalasenegative

Urease test

In presence of urease producing micro organisms, urea splits into ammonia and carbon dioxide. The development of the purplish pink color suggested the bacteria EBBO3 was urease negative.

Fig. 21 Bacteria EBBO 3 is urease negative

Oxidase test

The oxidase enzyme, present in the bacteria promotes the transfer of electrons to the methylene blue dye, turning it colorless. In this case the dark blue color persisted, confirming the bacterial strain EBBO3 to be oxidase negative.

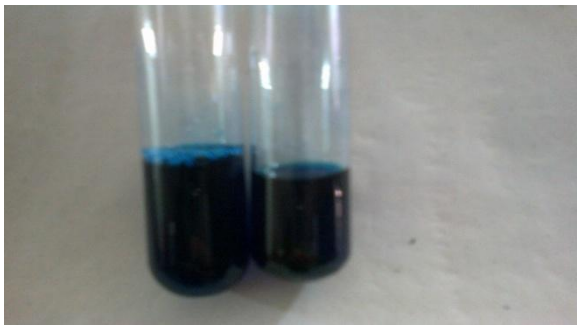

Fig 22. Bacteria EBBO3 shows negative oxidase test

Citrate utilization test

Citrate utilizing bacteria can grow on medium containing citrate as their sole carbon source. In case of bacterial growth, there is an increase in pH from 6.8, which in turn showed a change in the color of bromothymol blue. The formation of deep blue color after 48 hrs of incubation suggested a positive result.

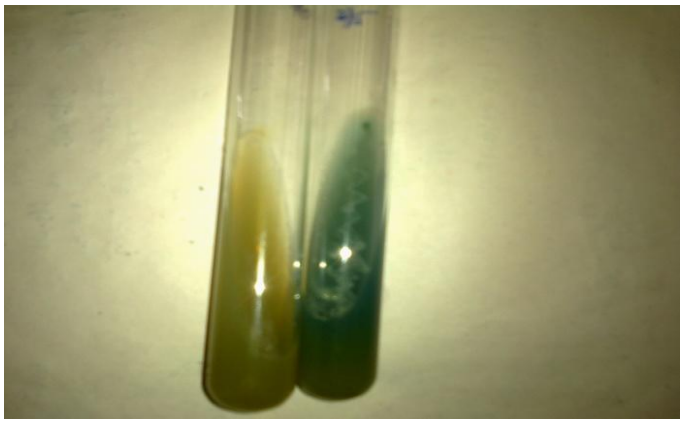

Fig 23. Bacteria EBBO3 shows positive citrate utilization test

M R Test

The test is used to detect the production of acid in the presence of glucose in the medium. In case of acid production the ph drops below 4.2, which can be detected by a change of color of medium on addition of phenol red indicator. The development of red color on addition of few drops of the indicator, confirms the test to be positive. In this case, the persistence of yellow color suggests a negative MR test.

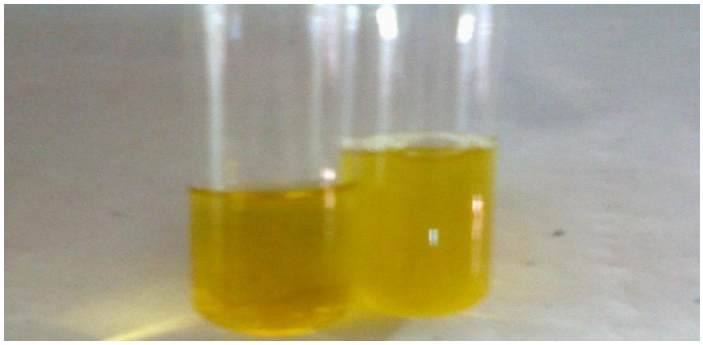

Fig 24Bacteria EBBO3 shows negative result towards MR test

Nitrate reduction test

The test determines the potential of bacteria to reduce nitrate to nitrite. The bacteria EBBO3 gave a negative result as there was no color change from the control on addition of sulfamilic acid and α-naphthyl amine mixture (1:1).

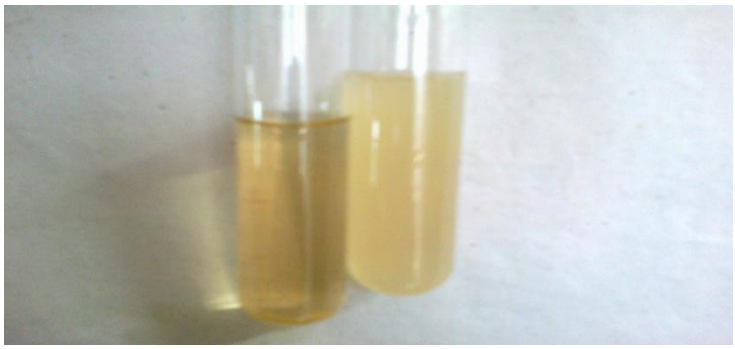

Fig. 25 On performing Nitrate Reductase Test strain EBBO3 gave negative result

Indole test

The test determines whether the bacterial strain produces the enzyme tryptophanase. The enzyme is responsible for the formation of indole from tryptophan. The formation of a red color on the top layer confirmed the production of tryptophanase by the bacterial strain EBBO3.

Fig 26. Positive result towards Indole test

V.P test

This test indicates the ability of bacteria to ferment carbohydrates (especially glucose). There was no crimson color development which indicated, strain EBBO3 is negative towards V.P test

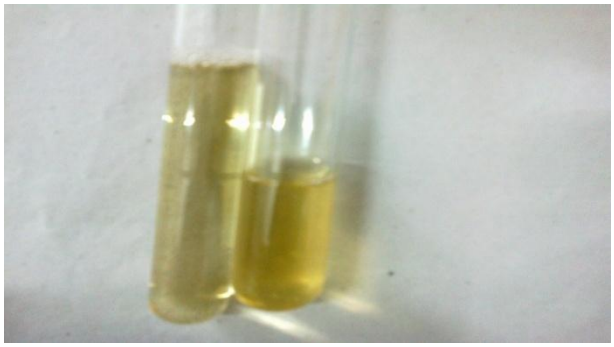

Fig 27. Strain EBBO3 showed negative result towards VP test.

Test for production of acid- gas using various carbon sources (carbohydrate metabolism)

This test detects the ability of the bacteria to ferment carbohydrate sources such as dextroxe and sucrose and produce acid and gas. On production of acid the color of the medium changes from blue to yellow. The presence of bubbles in the Durham's tube indicates gas production. The bacteria EBBO3 produces acid from both sucrose and dextrose. However, no gas production was recorded.

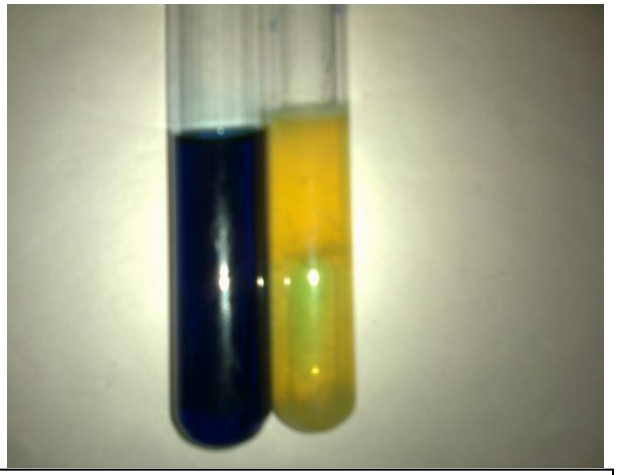

Fig.28. a. Bacteria EBBO3 shows a positive acid test and a negative gas production test when base was used

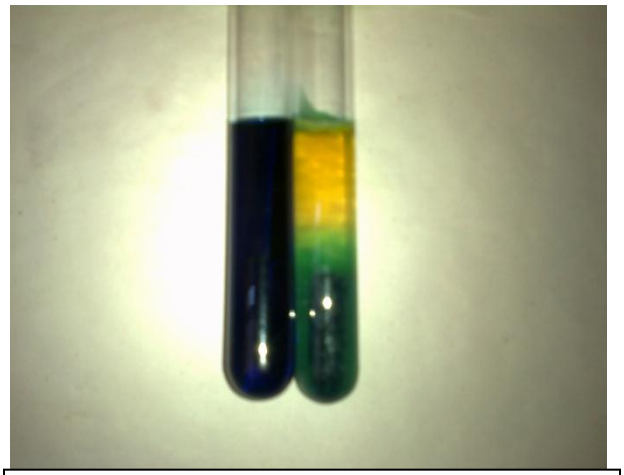

Fig. 28.b. Bacteria EBBO3 shows a positive acid test and negative gas production test when dextrose was used

H_2S gas production test

The test indicates the ability of bacteria to produce hydrogen sulfide gas from sulfur containing amino acids. In case of bacteria EBBO3, there was no black coloration of ferrous sulfide on the suspended lead acetate papers which indicates a negative H2S test.

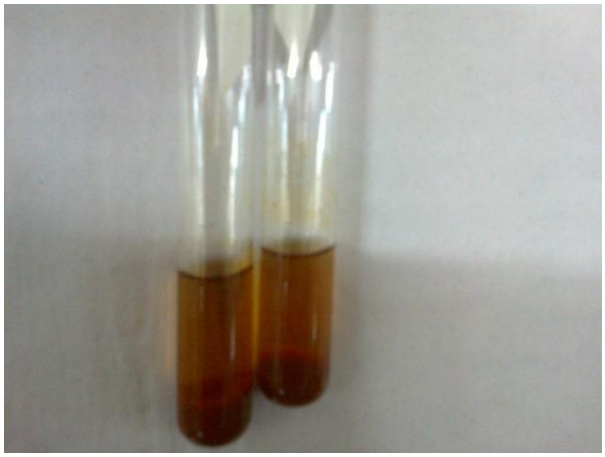

Fig 29. Bacteria acid shows no H2S gas production test

Starch utilization test

The appearance of brownish light colored patch around the colony indicates the bacteria EBBO3 gives a positive starch test. It is capable of hydrolyzing starch using amylase enzyme.

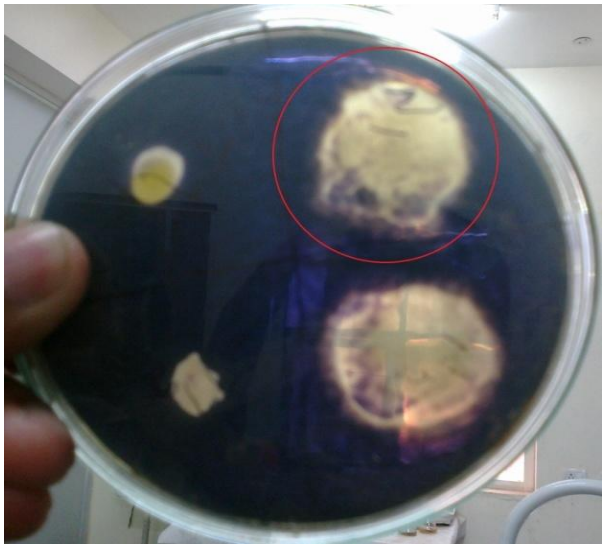

Fig. 30 Bacteria EBBO3 shows a positive starch utilization test

Antimicrobial assay

The formation of clear zone surrounding the wells containing antibiotics suggest the bacterial strain EBBO3 growth gets inhibited in presence of antibiotics such as amplicillin, ciprofloxacin and azythromycin.

Fig 31. Formation of clear zone around the wells suggest EBBO3 is sensitive to antibiotics.

Bacterial identification was performed in accordance with Bergey's Manual of Determinative Bacteriology and it was deduced that the test microorganism is a Gram positive, rod shaped, large and uniform *Bacilli.* Further characterization up to genus level can be confirmed through 16srRNA identification.

CHAPTER 5

CONCLUSION

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5. CONCLUSION

The present study deals with the removal of silica from bauxite ore of an aluminium industry by biological method. Bacterial colonies were successfully isolated and potential silica leaching strains was screened. Various process parameters such as pH, temperature, aeration time, inoculum size, age of the inoculum and bauxite percentage were studied through Taguchi method for process optimization. Optimum conditions for bioleaching of silica were obtained as pH 7.5, temperature 25ºC, initial aeration time of 30 min, bauxite percentage of 5% using 48 hours old 5% inoculums. Biological leaching results showed a maximum of 41% silica was recovered at the end of 25 days. The biochemical characterization of the most potential bacterial culture confirmed it is of *Bacillus* sp.

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